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HEAT EFFECTS ON A MARINE SNAIL

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The ability of intertidal prosobranch molluscs to survive high temperatures is related to their position in the intertidal zone and to their geographical distribution (Gowanloch and Hayes, 1926; Broekhuysen, 1940; Gunter, 1957; Southward, 1958; Fraenkel, 1961, 1966, 1968; Sandison, 1967; Newell, 1970; Markel, 1971). The high lethal temperature of *Littorina littorea* at Woods Hole, Massachusetts was 1–2° C lower in the winter than in the summer; also small differences exist between individual snails of the same species and these differences correlate with the relative locations of the individuals in the intertidal zone (Fraenkel, 1968). The heat tolerance of isolated tissues and enzymes correlates with the resistance of the intact animals and the heat resistance of tissues of many species are identical in summer and winter (Dzhamusova, 1960; Ushakov, 1964; Zhirmunsky, 1967). Intertidal prosobranchs are exposed to considerable temperature fluctuations during a tidal cycle, and often to great temperature variations seasonally. Thus *Littorina littorea* at Woods Hole is alternately frozen in the winter (Kanwisher, 1955, 1959, 1966) and heated by the hot summer sun. Even tropical snails may experience large temperature variations diurnally with tidal exposure and seasonally at the extremes of their ranges. For example, Lewis (1963) found tissue temperature differences in exposed specimens of *Nerita tesselata* in Barbados ranging from 6.0° C above to 14.0° C below ambient sea surface temperatures, and inshore water temperatures as low as 14.6° C and air temperatures of 11.2° C in February were found in the Florida Keys in an area near the 28.8° C isotherm for the maximum average annual sea surface temperature (Stephenson and Stephenson, 1950).

Mechanisms in prosobranchs compensatory for conditions of seasonal temperature fluctuations have been found in several species. For example, *Littorina littorea* can survive how temperatures better in the winter than in the summer (Kanwisher, 1966; Sömme, 1967). Segal (1965) reported that both high-level and low-level intertidal populations of the limpet, *Acmaea limatula*, had a faster heart rate in the winter at any given temperature than did summer animals. Effects of temperature on oxygen consumption also correlate with intertidal and geographic distribution (Newell, 1970; Sandison, 1967).

Ohsawa and Tsukuda (1956) noted a shift toward lower optimal temperatures for the extruding response of winter specimens of the periwinkle, *Nodilittorina granularis*. Except for *N. granularis*, few data exist dealing with the behavioral aspects of acclimation in prosobranchs.

Marine snails, when heated, typically enter a reversible state of heat coma several degrees below the temperature from which they fail to recover. This coma consists of detachment of the foot from the substratum and immobility, usually with

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the foot extended (Fraenkel, 1968; Sandison, 1967). The present study tests the effect of acclimation upon the temperature of heat coma in the common Atlantic Coast periwinkle, *Littorina littorea* which occurs primarily between high neap tides and low water spring tides. This study also compares thermal limits for foot reflexes and muscle contractions in cold and warm adapted specimens of *Littorina littorea*. These behavioral changes are correlated with changes that occur in the spontaneous activity of the central nervous system.

Materials and Methods

Specimens of *Littorina littorea,* obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts, were acclimated to different temperatures in ten gallon aquaria half-filled with artificial sea water (Instant Ocean) in rooms maintained at 5° C, 15° C, and 25° C. The snails were maintained on a constant 12 hour light cycle and were not fed during the period of acclimation. For measurements of heat coma, snails were removed from one of the constant temperature rooms and placed in a seawater bath of about 2.3 liters capacity at the temperature at which they had been acclimated. The temperature was then raised in stepwise fashion; the bath temperature was held constant for five minutes, and then was raised by 1° C; held for five minutes and then raised again. Temperature was controlled at each step to within less than 0.5° C by means of a knife-blade type heater controlled by a mercury thermostat and a "minitrol" relay. Uniformity of temperature throughout the bath was achieved by vigorously bubbling air through the water.

In order to ascertain how quickly a snail reaches temperature equilibrium with the bath, a thermistor probe was inserted through a small hole drilled through the shell of a *Littorina* of 1.8 cc volume and sealed to the shell. The snail was then placed in a bath of 6° C. As soon as the body of the animal reached temperature equilibrium with the bath, the snail was transferred to another bath at 21° C. In three trials the time required for the snail to reach equilibrium with the bath at 21° C was 57–60 seconds, giving an approximate rate of temperature rise of about 0.25° C per second.

Lethal temperatures were measured on snails collected in February and acclimated for from three weeks to several months at 0.5° C, 5° C, and 25° C. The snails were placed directly into sea water previously heated to a given temperature and kept there for one hour (Fraenkel, 1960). The bath was aerated throughout the experiment. At the end of one hour the animals were removed and returned to 15° C sea water to permit recovery. Those snails that subsequently reattached to the container and crawled up the side and out of the water were considered to have recovered. Snails which behaved in this way following subjection to high temperatures were kept for three weeks in aquaria and appeared to be normal at the end of this time.

The hypothesis of muscle failure as an explanation of heat coma was tested by a series of length-tension meaurements at different constant temperatures using muscle preparations from 5° C and 25° C acclimated snails. The columellar muscle was chosen for test purposes because it is the muscle that pulls the foot into the shell and is involved in the heat coma phenomenon through its failure to cause withdrawal of the foot while the animal is in heat coma.

To obtain a muscle preparation, the shell was cracked with a nutcracker to expose the body and to leave the attachment of the columellar muscle to the columella intact. The visceral mass was excised, the head slit open, and the organs of the head and foot removed, including all the major ganglia of the central nervous system. The shell remnant (columella) was then placed in a clamp attached to a Palmer stand, the muscle was attached to a photodiode mechano-electrical transducer by a 2 inch length of silk thread which was tied to a pin inserted through the operculum, and the preparation was immersed in sea water in a battery jar of 2.3 liters capacity and aeration was maintained. Sea water was an adequate medium for maintaining active muscle preparations for at least a week at 10° C. Electric stimuli were administered as monophasic square pulses of 15 volts, 20 msec pulse duration at 4 pulses per second from a Grass stimulator and isolation unit via chlorided silver electrodes which were inserted into the middle of the muscle mass, about 2 mm apart. The electrodes were insulated by three coats of spar varnish, with about one millimeter of the ends exposed. Isometric tension was amplified by a direct current amplifier and recorded by a Sanborn recorder, model 320. Temperature of the bath was held constant by means of a 125-watt knifeblade type heater and a mercury contact thermometer. Temperatures below room temperature were maintained by packing dry ice around the bath.

Contraction characteristics of the 5° C-acclimated and 25° C-acclimated snails were compared at several test temperatures over the range 5° C to 40° C. Standard length was taken to be the length at which the tetanic tension was greatest at each test temperature.

Expression of tension development as a function of cross-sectional area of the columellar muscle was complicated by the fact that the dimensions of the muscle cannot practically be determined, as this muscle becomes intermeshed with fibers from other muscles in a most intricate manner upon its entry into the foot. To obtain an approximation of the dimensions of the muscle, it was assumed that the thickness of the contracting muscle was uniform throughout its length, from the point of attachment on the columella to that on the operculum. The standard length of muscles from six snails was determined by progressively stretching the muscle, stimulating and recording tension development. As soon as peak tension was passed, the muscle was returned to the length at which peak tension was developed. At this length all excess flesh of the head and foot was excised, leaving a muscle strip of uniform diameter similar to that near the point of attachment to the columella. This strip was then weighed, and in all cases was found to be approximately 50% of the eviscerated, deganglionated muscle preparation. The weight of the columellar muscle was, therefore, taken to be 50% of the untrimmed muscle preparation in all animals.

Knowing the weight and the length of a muscle, the tension development in grams per square centimeter cross-sectional area was determined by the equations: 1) $P = \frac{F}{A}$ which equals $P = \frac{F}{v/l}$, rearrangement gives $P = \frac{Fl}{v}$; 2) also, $P = \frac{m}{v}$ and $v = \frac{m}{\rho}$. Substituting for v in equation 1 gives $P = \frac{Fl\rho}{m}$; assuming ρ is ap-

proximately equal to 1, then $P_0 = \frac{F_0 l_0}{m}$ where P = tension, A = cross-sectional area of the muscle, F = force, m = mass, ρ = density, l = length, v = volume, P_0 = tension developed at standard length, and l_0 = standard length of the muscle.

Spontaneous activity of the central nervous sytem of *Littorina littorea* was measured using a suction electrode of inside diameter 0.2 mm, into which the severed end of a nerve tract leading from a ganglion was drawn. Spontaneous action potentials were amplified by means of a Tektronix 122 low level preamplifier, time constant 0.2 seconds, and were displayed on a Tektronix 502 dual beam oscilloscope. Records were obtained photographically at a film speed of 100 mm per second.

A nerve preparation consisted of the intact circumeosophageal nerve ring containing the cerebral, pleural, and pedal ganglia from a snail previously acclimated to either 5° C or 25° C. This preparation was obtained from the snail by opening the head, removing the buccal mass, salivary glands, and gut, thereby exposing the ganglia. The nerve ring was then removed and placed in a small lucite chamber, the bottom of which was covered with paraffin wax. The preparation was held in position by a small tungsten staple which was slipped over the connectives and inserted in the paraffin. The preparation was bathed by a constant flow of sea water through the chamber from a large lucite reservoir maintained at constant temperature. Aeration and mixing in the reservoir were achieved by bubbling air through the sea water. All equipment used in these experiments except the oscilloscope and kymograph camera were enclosed in a Faraday cage. Temperature was monitored by a thermistor probe placed in the nerve chamber close to the ganglia. During most experiments the starting temperature was 5° C. This temperature was maintained for 15 minutes before recording the nervous activity. Kerkut and Taylor (1958) found that 3 to 5 minutes equilibration of the slug isolated ganglia was sufficient to obtain steady rates of spontaneous activity. This was confirmed in the present study and a 5 minute equilibration period was allowed at each experimental temperature before spontaneous activity was recorded. During the course of an experiment the temperature in the lower ranges was raised by 5° C increments. Preliminary experiments revealed a difference in the temperature of cessation of spontaneous activity in the 5° C-acclimated and the 25° C-acclimated ganglia; therefore, the temperature was raised by 1° C increments above 25° C for the 5° C-acclimated ganglia and above 30° C for the 25° C-acclimated ganglia, in order to establish the temperature of cessation of activity for each.

Initially, measurements were made of the spontaneous action potential frequency recorded from the largest of the pedal nerves from the pedal ganglion, but the gross spike frequency was very high, with the spikes tending to occur in bursts. It was subsequently found that the spontaneous activity of the tentacular nerve leading from the cerebral ganglion was of lower and more regular spike frequency. As cessation of activity in the two ganglia occurred at the same high temperatures, the right tentacular nerve was chosen for all subsequent experiments. A total of 14 experiments was performed in which 7 nerve preparations were from 5° C acclimated snails and 7 were from 25° C acclimated animals.

Results

Heat coma

A marine snail immersed in a seawater bath behaves in a characteristic manner when exposed to rising temperatures. This consists of ever increasing activity (crawling up the sides of the container), which begins to diminish at higher temperatures, finally resulting in complete cessation of motility. The snail's response to temperature change is quite rapid; an active snail placed directly at high temperatures becomes inactive within a few seconds. At the temperature of complete immobility the tentacles may still wave about. With further increase in temperature the foot begins to curl back away from the substratum, usually beginning at the anterior end. This process continues until the animal falls to the bottom of the container. An animal which may have been attached to the bottom of the vessel also releases its attachment. If the elevated temperature is maintained, the snail lies on the bottom of the vessel, usually with the foot fully extended, showing few, if any, spontaneous movements. In all experiments in this study the criterion for heat coma was taken to be the temperature at which the animal relinquished its hold upon the substratum. In this state the snail would usually withdraw in response to prodding, but reflex activity was abolished if the temperature was raised 3 to 4° C higher. If the temperature was then lowered, reflex activity returned within 10 to 15 seconds, and reattachment and apparently normal behavior returned in most individuals within 5 to 15 minutes.

The effect of acclimation upon the temperature of development of heat coma in *Littorina littorea* is shown in Table I. Some specimens of *Littorina* obtained from Woods Hole were acclimated at 5° C, 15° C, or 25° C for 14 days, others for 50–55 days. Attempts to acclimate *L. littorea* at 30° C failed because the snails invariably died after five to six days at this temperature. One group of *L. littorea* were acclimated for 14 days at 0.5° C. The heat coma temperatures increased from 30.5° C for 0.5° C acclimation to 38.8° C for 25° C acclimation.

Table I

The effect of acclimation temperature upon temperature of heat coma in Littorina littorea.

Temperature of acclimation	Acclimation time (days)	Number of snails	Mean temperature of heat coma *E.S.E.	t-test for significance of difference between means of 5° C- and 25° C-acclimated snails	Date snails were collected
0.5° C	14	10	30.5° C ± 0.81		Feb. 1968
5° C	14	10	32.5° C ± 0.53	$P < 0.001$	Apr. 1967
15° C	14	10	35.0° C ± 0.38		Apr. 1967
25° C	14	10	38.8° C ± 0.42		Apr. 1967
5° C	54	10	31.6° C ± 0.62	$P < 0.001$	Apr. 1967
15° C	55	9	35.9° C ± 0.70		Apr. 1967
25° C	50	10	38.9° C ± 0.41		Apr. 1967

* Estimated standard error.

TABLE II

Heat coma temperatures of Littorina littorea *taken directly from the sea at Woods Hole, Massachusetts.*

Date of collection	Number of individuals	Mean temperature of heat coma *E.S.E.	Temperature of sea water at start of experiment	t-test for significance of difference between means of December snails and July 18 snails
July 18, 19, 1966	10	37.6° C ± 0.16	22.5° C	$P < .05$
Dec. 20, 22, 1965	5	32.6° C ± 0.51	22.0° C	

* Estimated standard error of the mean.

Variance analysis of the data shows no significant difference between the heat coma temperatures of *L. littorea* acclimated for the two times of 14 and 50–55 days. Thus acclimation was complete by two weeks.

The reversibility of the shift in heat coma temperature was shown by an experiment in which ten *L. littorea* were acclimated at 25° C for two weeks, then returned to 5° C for two weeks. The mean heat coma temperature of the snails after two weeks acclimation at 5° C was 32.6° C. This agrees well with the heat coma temperature of snails that had remained at 5° C (Table I).

In Table II heat coma temperatures for summer and winter specimens of *L. littorea* are compared. At the time of collection of the summer animals in July, the ambient sea surface temperature at Woods Hole was 22.0° C to 22.5° C. The winter snails were shipped by air from Woods Hole to Chicago in December. The mean heat coma temperature of the winter snails is significantly below that of the summer animals; this provides evidence that acclimatization occurs seasonally in the sea.

Lethal temperatures

Fraenkel (1960, 1968) found for *Littorina littorea* that animals tested in March died after one hour at 39° C while summer animals died after the same time at 40–41° C. I found that snails acclimated to 5° C showed a survival rate of 7 out of 10 at 40° C and 1/10 at 41° C, whereas animals acclimated to 25° C showed a 5/10 survival at 41° C. Snails acclimated at 0.5° C when heated at 40° C for one hour had a survival rate of 7 out of 10 in each case. This shows that acclimation in the laboratory shifts the lethal temperature of snails by about the same amount as seasonal acclimation in the field as measured by Fraenkel (1968).

For any species of animal there are many "lethal temperatures" according to the measured time to death (Orr, 1955a). Thirty degrees Centigrade, constantly maintained, was a lethal temperature for *L. littorea* over the course of four to five days. Attempts to acclimate the snails slowly to high temperatures by keeping them at 25° C for varying lengths of time prior to placing them in the 30° C room consistently failed. As *L. littorea* can survive for months at 25° C, the lowest lethal temperature for this species in the laboratory lies between 25° C and 30° C.

Muscle measurements

The heat coma could be explained by failure of muscles to perform at high temperatures, by the inability of the nervous system to initiate muscular activity, or by both occurring simultaneously. As the temperature of heat coma has been demonstrated to shift approximately 8.5° C between the acclimation temperatures 0.5° C and 25° C in *Littorina littorea,* a change in the ability of muscles to respond to stimuli should occur as a result of acclimation if heat coma is caused by failure of muscles.

In both the 5° C-acclimated and 25° C-acclimated columellar muscles, the developed tension showed an approximately linear decline over the experimental range 5° C to 40° C (Figure 1) : there was no significant difference in maximum tension developed by 5° C- and 25° C-acclimated muscles at any given temperature ($P > .05$). This decline in muscle tension with increase in temperature could be the result of an increase in the rate of the relaxation processes and/or increased rate of decay of the active state. No measurable muscle contraction could be elicited from either warm or cold acclimated muscles at 40° C. Table III shows that both the time to peak tension and time to one-half relaxation decreased with increase in temperature.

A difference was noted in the relaxation rates of the 5° C- and 25° C-acclimated muscles when both were tested at 10° C, but not at 25° C (Table III and Fig. 2).

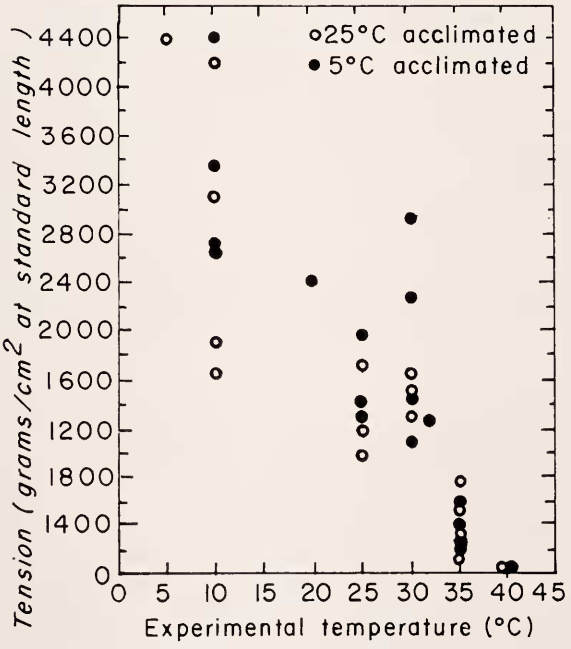

FIGURE 1. Maximum isometric tetanic tension of the columellar muscle of *Littorina littorea* acclimated at 5° C and 25° C measured at different experimental temperatures; open circles represent 25° C-acclimated, closed circles represent 5° C-acclimated muscles.

TABLE III

Comparison of the time to maximum tetanic contraction and to one-half relaxation of the columellar muscle of 5° C-acclimated and 25° C-acclimated L. littorea *at 10° C and at 25° C.*

Number of muscle preparations	Acclimation temperature	Mean time to peak tension *E.S.E.	Mean time to ½ relaxation	Test temperature	t test for significance of difference between means in relaxation data at 10° C
4	5° C	37.9 ± 2.6	35.5 ± 5.1	10° C	$P < 0.05$
4	25° C	27.1 ± 7.0	16.5 ± 4.2	10° C	
5	5° C	4.2 ± 0.4	4.3 ± 0.5	25° C	
3	25° C	4.7 ± 0.4	4.3 ± 0.7	25° C	

* Estimated standard error of the mean.

The time to maximum contraction was not significantly different, but the relaxation phase was significantly longer in the 5° C-acclimated muscles than in the 25° C-acclimated muscles.

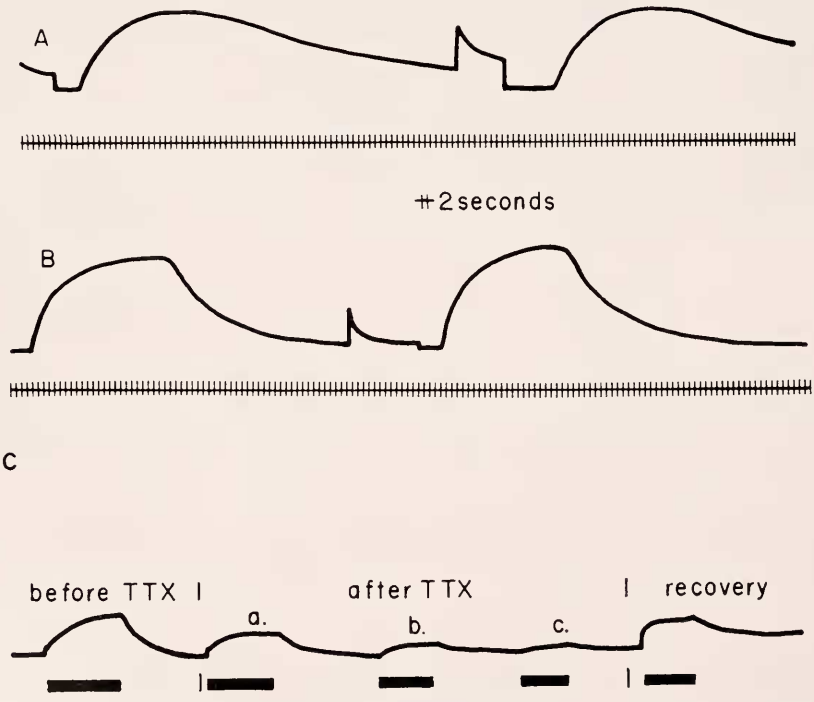

FIGURE 2. Contractions of *L. littorea* columellar muscle at 10° C showing the difference in time to one-half relaxation in (A) 5° C-acclimated muscle and (B) 25° C-acclimated muscles; (C) the effect of 1×10^{-5} gm/ml tetrodotoxin on isometric contraction of the columellar muscle of *L. littorea;* time after application of TTX; in (a) 12 minutes (b) 27 minutes, and (c) 37 minutes. Black bars indicate duration of stimulus.

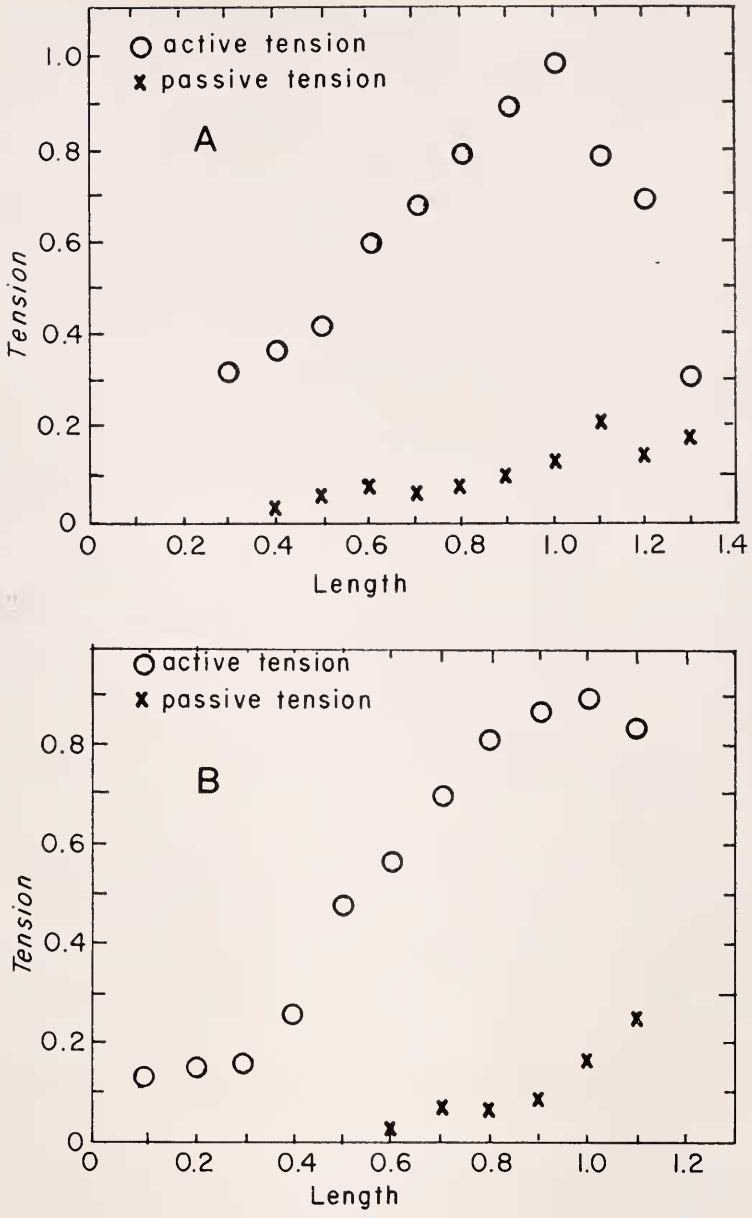

FIGURE 3. (A) Mean of three length-tension measurements of 5° C-acclimated *L. littorea* columellar muscles tested at 10° C. Open circle represents active tension, and X represents passive tension. (B) shows the mean of four length-tension measurements of 25° C-acclimated *L. littorea* columellar muscles tested at 10° C. Open circle represents active tension, and X represents passive tension.

Passive and active length-tension curves are presented in Figure 3 for measurements taken at 10° C. These were superimposable for muscles from 5° C-acclimated (Fig. 3A) and 25° C-acclimated (Fig. 3B) snails at 10° C and at all other temperatures of measurement. It is therefore unlikely that the difference in relaxation time in the 5° C- and 25° C-acclimated muscles at 10° C is caused by a change in the elastic elements of the muscle as a result of acclimation.

Whether or not acclimation temperature has any affect on the ability of the columellar muscle to contract after exposure of the snail to extreme temperatures was tested as follows. Specimens of *L. littorea* were acclimated at different temperatures for at least nineteen days. The intact animals were then placed in a seawater bath previously heated to a given high temperature and were kept at this temperature for one hour. At the end of an hour the snails were removed from the bath and the contractions of the columellar muscles were recorded.

The results of this experiment show that there is at most only a difference of 1° C in the heat resistance of the muscles of the 25° C-acclimated *L. littorea* compared to those of the 5° C-acclimated snails. Muscle response in snails acclimated at 5° C did not fail (10/10 responding) after being heated at 43° C for one hour, but failed (0/10) after one hour at 44° C. In muscles from animals acclimated at 25° C, there was no failure after one hour at either 43° C (10/10) or 44° (10/10), but failure of response was complete (0/10) after one hour at 45° C.

A possible role of the central nervous system in influencing muscle heat resistance was found in experiments in which isometric twitch responses of the columellar muscle of *intact* 5° C- and 25° C-acclimated *L. littorea* were compared at different high temperatures, starting at room temperature. Both the 5° C- and the 25° C-acclimated muscles failed to respond to electrical stimuli at 43° C. This was at least 3° C *higher* than the temperature of muscle failure in deganglionated preparations.

These experiments show that acclimation temperature of the snail does not change the heat resistance of the columellar muscle by more than 1° C to 2° C.

In the preceding experiments the muscle was stimulated electrically. There is histological evidence that a nerve plexus exists in the foot musculature of gastropods (Bullock and Horridge, 1965): this plexus may also be present in the columellar muscle. Several attempts were made by use of methylene blue and Bodian's stain to find a nerve plexus in the foot and columellar muscle but no positively identifiable nervous elements could be found other than large nerve tracts. The possibility exists, however, that electrical stimulation of the columellar muscle occurred indirectly *via* a plexus, or other peripheral nerves, rather than directly.

Failure of the muscle to contract at high temperatures might have been the result of a temperature effect upon the neuromyal junction or on the peripheral nerve rather than upon the muscle. In an attempt to ascertain whether or not the muscle was being directly stimulated, the following nerve-blocking drugs were applied: xylocaine, procaine, d-tubocurarine, hexamethonium, probanthine, tricaine-methane-sulfonate, and tetrodotoxin (TTX). The concentration of drug used in most instances was 1×10^{-4} g/ml except TTX which was used in a concentration of 1×10^{-5} g/ml. In order to enhance penetration of the drugs, the

muscles were usually trimmed until a strip of 2 millimeters thickness remained. In some experiments the preparation was exposed to the drug for several hours in order to ensure complete penetration of the drug. The preparations were kept at 10° C to prevent possible deterioration of the muscle; preliminary experiments had shown that these preparations could be kept in the cold for many hours without noticeable effect upon muscle contraction.

None of the drugs except tetrodotoxin (Fig. 2C) abolished the columellar muscle contraction. The abolition of contraction by tetrodotoxin tends to support the hypothesis that columellar muscle contraction is mediated by nerves, but since tetrodotoxin is known to block sodium conductance in some muscles as well as in nerves (Narahashi, Moore, and Scott, 1964) the question still remains whether the muscles ceased to contract because of nerve block or because the muscle was directly affected. Twarog (1967) found that tetrodotoxin in high concentrations had no effect upon spike amplitude or contraction of *Mytilus* anterior byssus retractor muscle (ABRM) bundles when the muscle cells were stimulated directly. Moreover, direct application of tetrodotoxin to the isolated ganglia of *L. littorea* blocked all spontaneous nervous activity within five minutes; activity subsequently returned upon washing the ganglia with sea water. From the absence of effects of the acetylcholine-antagonizing drugs, it appears that if stimulus of the columellar muscle is indirect, then the excitatory transmitter is possibly not acetylcholine.

Several deganglionated muscle preparations of *L. littorea* were held at 5° C for varying periods of time (up to several days) in an attempt to ascertain whether or not there might be a decline in the ability of the muscle to contract which might be attributable to nerve degeneration. The amplitude and other characteristics of contraction of the deganglionated muscle preparation held in the cold for six days were indistinguishable from those of a freshly dissected preparation. Several other preparations in which the pedal ganglia were left intact were held at 5° C for 15 days. At the end of this time the muscle preparation did not respond to electrical stimuli, although some spontaneous action potentials were recorded from nerves from pedal ganglia. In view of the fact that muscle contraction was unaffected in the deganglionated muscle after six days at 5° C and that neurones in the pedal ganglia survived after two weeks in the cold, it seems possible that the peripheral nerves may survive as long as the muscle fibers themselves, particularly if there are ganglion cells associated with the nerves. The failure of the muscle to contract after fifteen days in the cold could as well be attributed to loss of viability of the muscle cells as to nerve degeneration.

Spontaneous nervous activity in isolated ganglia

The previous section indicates that heat coma and its modification by acclimation are not the result of temperature effects on muscle. The hypothesis that heat coma depends upon effects in the central nervous system was tested by measurements of electrical activity in central ganglia at different temperatures.

Figure 4 gives the normalized mean spike frequency in a tentacular nerve at different temperatures from fourteen experiments. Figure 4 shows that spike frequency in both the 5° C-acclimated and 25° C-acclimated ganglia increases with increase in temperature up to about 20° C, and declines rapidly above 25° C.

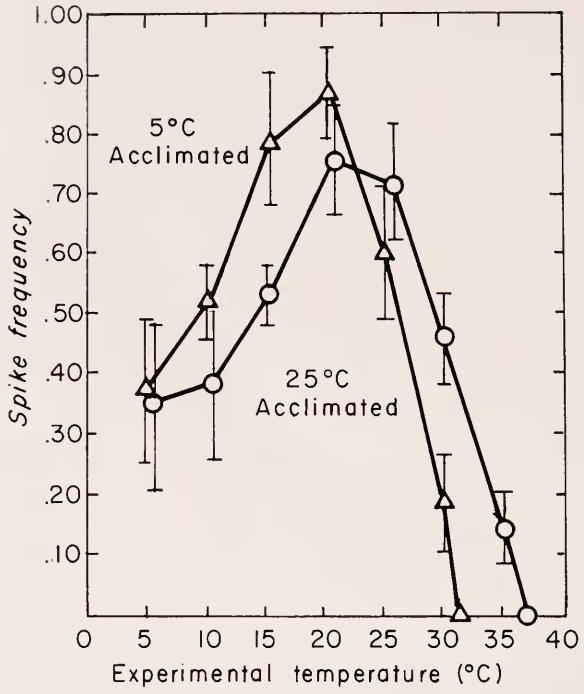

FIGURE 4. The effect of acclimation temperature upon spontaneous activity of isolated ganglion from *L. littorea:* open triangle represents 5° C-acclimated, open circle represents 25° C-acclimated. Range marks indicate standard error of the mean. Frequency is plotted as the ratio of spike activity at a given temperature to the maximum activity exhibited by the preparation over the experimental range. Each point is the mean of 7 experiments.

There is no statistical difference between the frequency of the 5° C-acclimated and 25° C-acclimated ganglia up to 25° C. However, at 30° C the spike frequency of the 5° C-acclimated ganglia is approximately half that of the 25° C-acclimated ganglia. The mean temperature of complete (but reversible) cessation of spontaneous activity for the 5° C-acclimated snails was $31.4 \pm 0.5°$ C, while that of the 25° C-acclimated snails was $37.1 \pm 0.8°$ C. The t test indicates a difference between these means at the 0.005 level of significance. Lack of hysteresis in the temperature effect upon spontaneous activity of the ganglia was shown in that the gross spike frequency at 18° C during cooling after heating to over 31° C was similar to that at 20° C during the rising phase of the temperature experiment.

In a few cases a large repeating unit could be followed, and analysis of these records shows that the increase in gross spike frequency with increase in temperature was the result of both an increase in the rate of firing of the repeating unit and the addition of other units. A plot of the activity of the repeating unit in two 5° C-acclimated ganglia and two 25° C-acclimated ganglia is shown in Fig. 5. There appeared to be no discernible difference between the activity of the repeating units resulting from acclimation except in the temperature of cessation of activity.

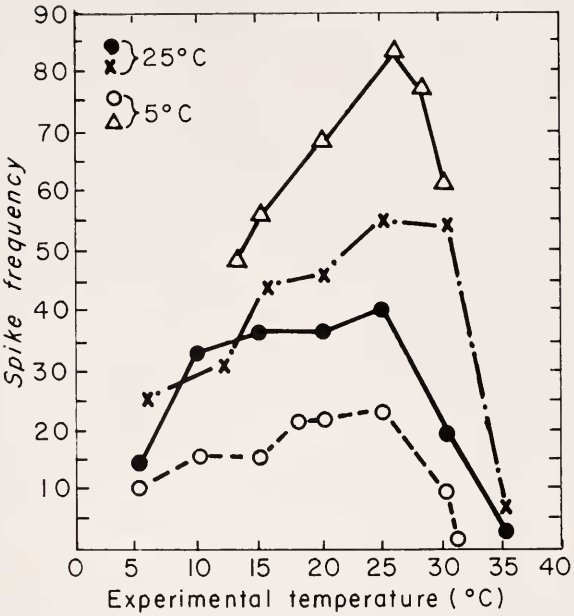

FIGURE 5. Comparison of the frequency of firing of 4 repeating units recorded from the tentacular nerve from two 5° C-acclimated snails and two 25° C-acclimated snails.

DISCUSSION

Littorina littorea is capable of coping with both long-term and short-term changes in environmental temperature. In the present investigation this capability is expressed by a shift in the temperature of heat coma as a result of laboratory acclimation to different temperatures.

Fraenkel (1968) found that *Littorina littorea* apparently has little ability to adapt seasonally in regard to lethal temperature, and a shift of only about 1° C was found for the same species as a result of temperature acclimation in the laboratory in the present study. It is difficult to imagine any adaptive significance in such a small shift in lethal temperature. However, *L. littorea* spends much of its time out of water and is exposed to a much greater range of temperatures than are organisms which show seasonal shifts in lethal temperatures, *e.g.*, fish (Prosser, 1973). The ecological significance of any given "lethal temperature" for a species depends upon whether or not the animal may be exposed to this temperature long enough to be killed or injured to the point where it might fall easy prey to some predator or disease, or suffer damage to the reproductive tissues. It is quite likely that *L. littorea* may survive body temperatures of over 30° C for a period of several hours on hot summer days. Maximum snail body temperatures recorded in the field are usually lower than the maximum tolerated in laboratory tests (Markel, 1971).

It is reasonable to suppose that in intertidal snails the lethal temperature(s) might be set at the genetic limit for the species, and if this limit were sufficiently high in the snail's environment, selective pressures would be exerted upon adapta-

tions that would allow the animal to cope with short-term sublethal temperature stress. A shift in the heat coma threshold would be an example of such an adaptation. The temperature of heat coma found for *L. littorea* was approximately 10° C lower than the lethal temperature. Similar range between coma and lethal temperatures was observed for *L. littorea* from Wales (Sandison, 1967) and for *Monodonta lincata* from the Mediterranean (Micallef, 1968). Coma temperature was more subject to change by acclimation than lethal temperature, hence lethality may involve defects in more and different physiological systems than does coma.

The possibility that heat coma in *L. littorea* may be caused by a direct effect of temperature upon the columellar muscle has been ruled out by the series of experiments in which muscles continue to contract at temperatures above lethal and coma levels and in which the maximum tetanic tension developed by muscles from 5° C- and 25° C-acclimated snails was shown to be not significantly different over the range 5° C to 40° C. These data support the results of Dzhamusova (1960), who found that deganglionated foot muscle preparations of *L. littorea* failed to respond to electrical stimuli after approximately 2 to 3 minutes at 40° C.

The data of this paper are in general agreement with the results of Dzhamusova (1967), Ushakov (1964), and Zhirmunsky (1967), who found that the heat resistance of molluscan muscles was constant within a species regardless of the thermal history of the individuals tested. In two species of *Littorina* Dzhamusova found identical heat resistance in populations taken from 10° C to 12° C waters in the Sea of Okhotsk and from 20° C to 23° C waters in the Sea of Japan.

The measurements of tension development of deganglionated muscle of *L. littorea* agree with the results of Cambridge, Holgate and Sharp (1959) who found a decrease in isometric contractions in *Mytilus* anterior byssus retractor muscle with increase in temperature but these measurements differ from those of Benthe (1954) who found that isotonic twitch contractions of the isolated foot of 3° C-, 12° C-, and 21° C-acclimated *Limnaea stagnalis* increased in amplitude over the experimental range 5° C to 20° C. However, it is difficult to see how Benthe could make valid comparisons of twitch amplitude since his measurements were not based upon tension developed per cross-sectional area of muscle.

Benthe (1954) found that the presence of central ganglia apparently imparted greater heat resistance of *L. limnaea* foot muscle than was exhibited by deganglionated preparations. This observation was supported by a number of experiments in the present study. In intact specimens of 5° C- and 25° C-acclimated *Littorina littorea* isometric twitch responses of the columellar muscle failed at 43° C, a temperature at least 3° C higher than that at which deganglionated muscle preparations failed to respond. Increased heat resistance conferred upon the muscle by central ganglia may explain in part the differences in results obtained by Dzhamusova (1960), who found the "maximum heat of resistance" of deganglionated muscle of *L. littorea* to be about 34° C and those of Fraenkel (1968), who heated intact snails of the same species for one hour and found a maximum temperature of 43° C to 44° C for the opercular reflex.

In the length-tension and contraction experiments it was not known whether the columellar muscle was responding directly to electrical stimuli or indirectly *via* nerves. Attempts to discover the mode of muscle stimulation by means of drug experiments were inconclusive, but a number of experiments reported in the

literature have shown that at high temperatures the neuromyal junction ceases to function at lower temperatures than either the muscle or the nerve, as in skate (Battle, 1926) and frog (Orr, 1955b; Jensen, 1972). It is possible that if stimulation of the columellar muscle of *L. littorea* occurred indirectly *via* motor nerves, failure of the muscle to contract at high temperatures may have been the result of failure of the neuromyal junction.

The heat coma phenomenon shows a good correlation with the temperature of cessation of spontaneous activity of the central nervous system as measured *in vitro*. The mean heat coma temperature for *L. littorea* acclimated at 5° C is 32.5 ± 0.5° C and the mean temperature of cessation of spontaneous activity of the isolated ganglia of snails acclimated at the same temperature is 31.4 ± 0.6° C; the heat coma temperature for snails acclimated at 25° C is 38.9 ± 0.4° C, while the mean temperature of cessation of CNS activity is 37.1 ± 0.8° C. It seems reasonable to infer that there is a causal relationship between the two phenomena in that heat coma is the visible expression of cessation of spontaneous nervous activity. It may be concluded that as in other poikilotherms [crayfish, teleosts (Prosser, 1973)] the nervous system of *Littorina* is most vulnerable to thermal extremes.

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Summary

Littorina littorea enters a reversible state of heat coma at temperatures below the lethal temperature of the species. The threshold of heat coma can be shifted as much as 8.5° C by acclimation; a linear relation exists between heat coma temperature and acclimation temperature over the experimental range.

Deganglionated columellar muscle preparations of 5° C and 25° C acclimated *Littorina littorea* showed an approximately linear decline in tetanic tension when tested from 5° C to 40° C. There was no statistically significant difference in the amplitude of muscle contraction or of tension-length curves of 5° C- and 25° C-acclimated muscles at any experimental temperature; this excludes failure of muscle contraction as the cause of heat coma. Speed of contraction was similar and relaxation slower in muscles from 5° C-acclimated snails than in muscles from 25° C-acclimated snails when measured at 10° C but not at 25° C.

Attempts to ascertain by the use of drugs and nerve degeneration experiments whether muscle contraction was elicited by direct or indirect stimulation were inconclusive, although the effects of TTX were consistent with the hypothesis that stimulation was by nerves.

Lethal temperature in *L. littorea* shows a shift of only about 1° C to 2° C as a result of temperature acclimation. The ability of the columellar muscle to

contract following exposure of the snail to extreme temperatures for one hour was similarly shifted only 1° C to 2° C by acclimation.

Measurement of spontaneous activity of the central nervous system of *L. littorea* showed good agreement between temperature of cessation of spontaneous activity and temperature of heat coma in 5° C- and 25° C-acclimated snails. Analysis of nervous activity showed that increase in activity with rising temperature was based upon both a greater frequency of firing of single units and activation of new units. Single unit frequency appeared to show no acclimation effect except in the temperature of cessation of activity.

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